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Award Number: DAMD17-02-1-0567

TITLE: Effects of Naturally-Occurring Estrogen-Fatty Acid Esters on Mammary Cell Growth and Carcinogenesis in Female Rats

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REPORT DATE: June 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2004	Annual Summary (1 Jun 2003 - 31 May 2004)

4. TITLE AND SUBTITLE
Effects of Naturally-Occurring Estrogen-Fatty Acid Esters on Mammary Cell Growth and Carcinogenesis in Female Rats

5. FUNDING NUMBERS
DAMD17-02-1-0567

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Research work completed thus far: (1) We have already evaluated the stimulatory effects of estradiol-17β-stearate on the growth of mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. Their effects have been compared with the effects of unesterified estradiol. (2) We have also determined the circulating levels of prolactin, FSH, and LH in all the animals. Right now we are still studying the growth-stimulatory effects of estradiol-17β-stearate and unesterified estradiol in pituitary, thymus, and liver of female rats. (3) We have completed comparing the carcinogenic activity of estradiol-17β-stearate and estradiol mammary vs. uterine cells in female ACI rats. We have also compared and correlated the differential carcinogenic activity of these two classes of estrogens with their differential growth stimulatory effects in various target organs.

The results of our studies sheds light on the physiologic and pathophysiologic roles of endogenously-formed estrogen-fatty acid esters in the body. Also, our findings may lead to the development of new strategies to mammary cancer prevention through inhibition of esterase-catalyzed release of bioactive estrogen.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
7

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

This is the annual progress report for the Predoctoral Traineeship Award that was originally awarded to Laura H. Mills in early 2002. Around June 2003, the PH.D. Dissertation Committee of Ms. Laura Mills approved her for graduation with a PH.D. Degree. At the request of my supervisor (Professor Bao Ting Zhu), I was named as the replacement P.I. on this grant proposal shortly following Ms. Mills' departure from the project (due to her graduation). After I had carefully read the proposal originally prepared by Dr. Laura Mills, I informed my supervisor that I would be very interested in continuing the studies described in Dr. Mills' proposal. A few months ago, I was approved to serve as the replacement P.I. on this project.

Body

The Specicific Aims of the Original Proposal:

I hypothesize that the naturally-occurring estrogen-fatty acid esters may be an especially important group of endogenous estrogens in stimulating cell growth and in inducing tumor formation in the mammary tissues as compared to the uterus. The estrogen-fatty acid ester may serve as a reservoir for the sustained release of 17β-estradiol in mammary glandular cells because they are surrounded by large amounts of adipocytes (which can be a storage site for estrogen esters). In partial support of this hypothesis, my recent study in Dr. Zhu's lab showed that treatment of female rats with E_2-17β-stearate (0.5 or 5 nmol/day) showed a much stronger growth-stimulatory effect on mammary glandular cells than on the uterine endometrial cells (determined by BrdU incorporation) (39, see Appendix 1). In contrast, E_2 at the same dose showed a stronger growth-stimulatory effect on the uterine cells than on the mammary cells (39). These data revealed, for the first time, that E_2-17β-stearate has a differential, strong hormonal effect in the fat-rich mammary tissues, and this effect was not observed with E_2.

To test this interesting novel hypothesis, I propose the following three specific aims:

Aim 1: I will evaluate the stimulatory effects of E_2-17β-stearate and 4-hydroxyestradiol-17β-stearate, two representative estrogen-fatty acid esters, on the growth of mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. Their effects will be compared with the effects of unesterified E_2 or 4-hydroxyestradiol. The circulating levels of prolactin, FSH, and LH will also be determined in all animals, which will serve as an indicator of estrogen's action on the pituitary. During these animal experiments, I will also collect the pituitary, thymus, and liver, and if time allows, and the growth-stimulatory effects of esterified vs. unesterified estrogens in these organs will be determined and compared with their effects in the fat-rich mammary tissue.

Aim 2: I will evaluate the carcinogenic activity of E_2-17β-stearate and 4-hydroxyestradiol-17β-stearate on mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. The results will be compared and correlated with the growth stimulatory effects determined under Aim 1.

Aim 3: I will evaluate the activity of estrogen esterase (the enzyme that hydrolyzes estrogen-fatty acid esters to release bioactive estrogens) in the mammary tissue, and its activity will be compared with that found in other organs such as the uterus and liver. These analyses will provide insights into our understanding of the mechanisms underlying a

preferential mitogenic action (and perhaps carcinogenic action) of estrogen-fatty acid esters in the breast over the uterus.

I believe that my proposed studies will provide a preliminary evaluation of the hormonal and carcinogenic actions of a representative fatty acid ester of E_2 and 4-hydroxyestradiol in the breast vs. the uterus in female Sprague-Dawley rats. The results of our studies will shed light on the physiologic and pathophysiologic roles of endogenously-formed estrogen-fatty acid esters in the body. Also, if my proposed animal studies demonstrate that various endogenous estrogen-fatty acid esters are stronger and more selective than the unesterified parent hormones in stimulating cell growth and in inducing tumor formation in the fat-rich mammary tissues, this data will form the basis for future epidemiological studies to determine the unique importance of endogenous estrogen-fatty acid esters in human breast cancers. The results of my studies may also lead to the development of new strategies to mammary cancer prevention through inhibition of esterase-catalyzed release of bioactive estrogen.

Research completed thus far:

1. We have already evaluated the stimulatory effects of E_2-17β-stearate on the growth of mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. Their effects have been compared with the effects of unesterified E_2.

2. We have also determined the circulating levels of prolactin, FSH, and LH in all the animals. Right now we are still studying the growth-stimulatory effects of E_2-17β-stearate and unesterified E_2 in pituitary, thymus, and liver of female rats.

3. We have completed comparing the carcinogenic activity of E_2-17β-stearate and E_2 mammary vs. uterine cells in female ACI rats. We have also compared and correlated the differential carcinogenic activity of these two classes of estrogens with their differential growth stimulatory effects in various target organs.

I intend to continue the studies described in Ms. Mills original research proposal. Specifically, I have planned to do the following:

1. I will continue to evaluate the carcinogenic activity of 4-hydroxyestradiol-17β-stearate on mammary vs. uterine cells in female Sprague-Dawley rats.

2. I will continue to evaluate the activity of estrogen esterase (the enzyme that hydrolyzes estrogen-fatty acid esters to release bioactive estrogens) in the mammary tissue, and its activity will be compared with that found in other organs such as the uterus and liver.

Key Research Accomplishments

1. Our results showed that chronic treatment of ovariectomized female rats with 0.5 or 5 nmol/day of estradiol-17β-stearate for 10 or 23 days had a stronger stimulatory effect on mammary glandular cell proliferation than treatment with equimolar doses of estradiol-17β. In the uterus, however, estradiol-17β was more active in stimulating the proliferation of uterine endometrial cells than estradiol-17β-stearate at equimolar doses.

Our results demonstrated, for the first time, that a naturally-occurring estradiol-17β-fatty acid ester has a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with estradiol-17β.

2. We found that none of the control ACI female rats developed mammary tumor and all lived healthily during the 12 month long carcinogenesis experiment. Among the 26 animals receiving an E_2 pellet, all the animals developed a large pituitary tumor (average weight = 254 ± 83 mg), but only 8 of them developed mammary tumor(s). A majority of the animals died or had to be terminated early because of severe sickness, due to the presence of the large pituitary tumor. Among the 26 animals implanted with the same molar dose of an E_2-fatty acid ester preparation (containing 63% E_2-17β-stearate and 37% E_2-17β-palmitate), a total of 9 animals developed mammary tumor, and none of the animals died of non-breast cancer-related sickness. Only 3 animals (11.5%; all with mammary tumor) developed a pituitary tumor, with a slightly smaller average size (173 ± 75 mg).

Reportable Outcomes

Listed below are papers and abstracts that have come out of this award. The names of the original P.I. (Laura H. Mills) and the replacement P.I. (Won Jun Lee) are highlighted.

Mills LH, Lee AJ and Parlow AF and Zhu BT [2001] Preferential growth stimulation of mammary glands over uterine endometrium in female rats by a naturally occurring estradiol-17β-fatty acid ester. Cancer Research 61: 5764-5770.

Lee AJ, **Mills LH**, Kosh JW, Conney AH and Zhu BT [2002] NADPH-dependent metabolism of estrone by human liver microsomes. Journal of Pharmacology and Experimental Therapeutics 300: 838-849.

Hook LL (Maiden name for Laura H. Mills), Lee AJY and Zhu BT [2000] Differential stimulatory actions of estradiol-17β-stearate on the growth of rat mammary vs. uterine cells. Proceedings of the American Association for Cancer Research 41: 429, San Francisco.

Mills LH and Zhu BT [2001] Chronic administration of 17β-estradiol inhibits intramammary lymphocyte proliferation in female rats. Proceedings of the American Association for Cancer Research 42: 237, New Orleans, Louisiana.

Mills LH, Sowell JW, Chapman JM and Zhu BT [2002] Synthesis of 4-hydroxyestradiol-17β-stearate, an estrogen fatty acid ester, and its stable prodrug 4-hydroxyestradiol-3,4-diacetate 17β-stearate. The South Eastern Regional Meeting of the American Chemical Society (SERMACS), November 13-16, 2002, Charleston, South Carolina.

Mills LH, Lee AJ and Zhu BT [2003] Naturally-occurring estradiol-17β-fatty acid ester, but not estradiol-17β, preferentially induces the development of mammary tumor in female ACI rats. Proceedings of the American Association for Cancer Research 44: 835-836.

Lee WJ and Zhu BT [2004] Strong inhibition of DNA methylation by caffeic acid and chlorigenic acid, two polyphenolic components present in coffee. Proceedings of the American Association for Cancer Research 45, Orlando, Florida.

Conclusions

1. Our results demonstrated, for the first time, that a naturally-occurring estradiol-17β-fatty acid ester has a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with estradiol-17β.

2. While E_2 was far stronger in stimulating the formation of pituitary tumor (100% incidence) than mammary tumor (30.7% incidence), E_2-fatty acid esters had a somewhat stronger activity than E_2 in inducing the mammary tumor but only had very weak activity in inducing pituitary tumor. Our data suggest that the endogenous E_2-fatty acid esters are pathophysiologically more important than E_2 for the *selective* induction of mammary tumor formation.

References

Mills LH, Lee AJ and Parlow AF and Zhu BT [2001] Preferential growth stimulation of mammary glands over uterine endometrium in female rats by a naturally occurring estradiol-17β-fatty acid ester. Cancer Research 61: 5764-5770.

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Appendices

Not included.